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BEHAVIORAL RESPONSES OF *BALANUS IMPROVISUS* NAUPLII TO LIGHT INTENSITY AND SPECTRUM

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Although barnacle larvae have been used in numerous classical studies on phototaxis and spectral response, results have been predominantly qualitative in nature. Groom and Loeb (1890), Ewald (1912), and Rose (1925) studied phototaxis in stage I–II nauplii of *Balanus perforatus*. Vischer and Luce (1928) attempted to define the spectral sensitivity of cyprid *Balanus amphitrite* and "*B. improvisus*". Barnes, Crisp, and Powell (1951) demonstrated orientation to light during settlement of cyprid *Semibalanus balanoides* and *Balanus crenatus*. Based on these and other studies, Thorson (1964) characterized barnacles as maintaining positive phototaxis throughout their larval life.

More recent studies have begun to quantify light responses of barnacle larvae. Barnes and Klepal (1972) determined the spectral sensitivity and threshold of photo-response at 522 nm for stage I nauplii of *Elminius modestus* and *S. balanoides*. Crisp and Ritz (1973) studied the effects of dark-adaptation on relative light sensitivity for stage II *E. modestus* and *S. balanoides,* and limiting intensities of white light for photo responsiveness of stage II *S. balanoides* and *B. crenatus* and cyprid *S. balanoides*.

With the exception of a series of studies on the zoea of the estuarine decapod crustaceans (Forward, 1974; Forward and Costlow, 1974; Forward, 1976b, 1977; Forward and Cronin, 1978), quantitative phototactic and spectral studies of larval crustacean groups are sparse (see Forward, 1976a; Aiken and Hailman, 1978).

In this study, short-term phototaxis (direction) and orthophotokinensis (velocity) of *Balanus improvisus* nauplii are investigated using a new method of video-computer quantification which greatly facilitates studies of movement in small organisms (Davenport, Culler, Greaves, Forward, and Hand, 1970). The ability to rapidly quantify and compare movement paramenters makes this system a potentially powerful tool for behavioral biassays (Anderson, 1971; Olla, 1974). Thus this study demonstrates the capabilities of this system and provides a rigorous analysis of the photobiology of barnacle larvae.

Materials and Methods

Experimental animal

Balanus improvisus nauplii were sorted from surface plankton tows taken at Pettaquamscutt River near Narragansett, Rhode Island. During the collection period (24 Oct–28 Nov., 1977) *B. improvisus* nauplii were abundant and easily

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sorted from plankton samples (Lang, 1979). Water temperature and salinity at collection times ranged from 7 to 14° C, 13 to 20‰. Nauplii were immediately transferred to filtered sea water at 15° C, 15‰ and placed in either 15° or 20° C temperature boxes with constant illumination. For experiments conducted at 30‰, the temperature of the 15‰ water was first allowed to equilibrate at 20° C and nauplii were then transferred to 5‰ salinity increments at one hr intervals.

All larvae were maintained overnight (8–12 hr) at specified temperature/salinity before being used experimentally. *Isochrysis galbana* and *Tetraselmis suecia* were added as food. The following morning, nauplii of the desired stage were sorted from initial cultures and groups of 20 transferred to 5-ml beakers. Light-adapted nauplii were exposed to room lights supplemented with a 60-W incandescent bulb for at least 1 hr prior to experimentation; dark-adapted nauplii were kept in dark temperature-controlled boxes for at least 1 hr before experimentation. Studies with other arthropods indicate that these times are adequate for light and dark adaptations (*e.g.*, Hamdorf and Schwemer, 1975; Barnes and Goldsmith, 1977). To minimize effects of possible larval diurnal cycles (Singarajah, Moyse, and Knight-Jones, 1967), all experiments were conducted between 13:00 and 16:30 hr. All nauplii were tested within 26 hr of capture.

Light stimulus and video system

A microscope and closed circuit television system were used to monitor and record swimming behavior of nauplii. Dark field substage illumination, interference filtered to 830 nm (about 15 nm half width), provided light for a Cohu 4400 television camera mounted on a Wild M-5 microscope body (Lang, Lawrence, and Miller, 1979). Larvae were placed in a 1.2 × 1.2 × 1.0 cm lucite cuvette filled to 0.5 cm depth. Movement was monitored in the horizontal plane.

A light stimulus presented horizontally and perpendicular to the cuvette wall was provided by two sources. For initial studies on light response of nauplii, a grating monochromater with 150-W xenon short arc lamp (Oriel Corporation) was used. For spectral and intensity studies a slide projector with 300-W tungsten bulb and thin film absorption filters (Ditric Optics) ranging from 440 to 640 nm in 20-nm intervals (about 7 nm half-width) was used (Latz and Forward, 1977). In both cases light intensity of quantum levels were regulated by neutral density filters. Intensity was measured by a YSI model 64A radiometer.

Experimental procedure

Experiments were conducted in a temperature-controlled, darkened room. A preparation of 20 nauplii was transferred from a 5-ml beaker to the test cuvette; the cuvette was then aligned on the dark field stage. Light-adapted nauplii were transferred under room lights; dark-adapted nauplii were transferred under dim light (interference filtered at 700 nm). All lights were extinguished and nauplii were allowed at least 30 sec prior to experimentation to recover from movement of the test cuvette during placement on stage.

For initial studies on naupliar response to light stimulus, five intensities of 480-nm light ranging from 27 to 0.0027 W/m^2 were used. Replicate preparations of nauplii were tested at each intensity in ascending order with 45-sec intervals between 2.5 sec stimuli. The response of nauplii 2.5 sec prior to stimulation, during stimulation, and 3.0 sec following stimulation were analyzed by computer.

For spectral response, the 830-nm substage light was turned on 5 to 15 sec prior to light stimulus, a 2.5-sec stimulus applied, and the substage light extinguished. After 1 minute, a second wavelength stimulus (in ascending order) was applied. After four exposures the preparation of dark-adapted nauplii was changed. For both spectral and intensity studies, computer analysis was limited to naupliar response during the latter 2.0 sec of the 2.5-sec stimulus.

The general procedure for testing response to light intensities was similar. One preparation of nauplii was exposed to a complete sequence of seven intensities of 480-nm light starting with an intensity estimated as subthreshold. All stimuli were 2.5 sec in duration with 30- to 40-sec intervals between each stimulus.

At least three replicates were run with each plankton sample. With the exception of stage III nauplii, at least two different plankton samples were represented.

Computer analysis

Video recordings of naupliar response to light stimuli were played back through a video-to-digital processor, the "Bugwatcher" (Greaves, 1975). Outlines of each nauplii within the camera field of view are delimited as X–Y coordinates. For this study, video tapes were analyzed by computer at 10 frames per second; every sixth frame of the normal 60 f/sec recording was fed into a Data General Eclipse S/200 computer. Time series of X–Y coordinates (video files) indicating displacement of nauplii at 0.1-sec intervals were generated for each replicate sample. A tone generator synchronized with the light stimulus shutter control marked periods of stimulation; a tone detector in the Bugwatcher determined light stimulus duration to the nearest 0.1 sec on video files.

Video files were analyzed using second generation programs developed by Wilson and Greaves (1979). Processed video files (see Greaves, 1975) yielded a time-scaled computer track of naupliar movement (Fig. 1A). About 10 to 15 nauplii were tracked simultaneously. Linear velocity (Fig. 1B) and direction of travel (Fig. 1C) were calculated for each 0.1-sec interval of the tracks; mean values for individual nauplii during a given time interval were determined (Fig. 1D). Mean direction of travel (DOT) and mean linear velocity (MLV) for all nauplii of an experimental group were then pooled to calculate sample means. Sample DOT distributions were tested using a Chi-square test (Batschelet, 1965). Standard SPSS (Nie, Hull, Jenkins, Steinbrenner, and Bent, 1975) and SAS (Bar, Goodnight, Sall, and Helwig, 1976) analysis of variance programs were used to test sample MLV data.

RESULTS

Initial studies on stage II naupliar light response

Preliminary observations indicated that stage II *B. improvisus* nauplii were strongly positively phototactic to lower light intensities (about 10 W/m^{-2}), particularly near 500 nm. The following experiments were devised to further characterize the responses of light-adapted nauplii.

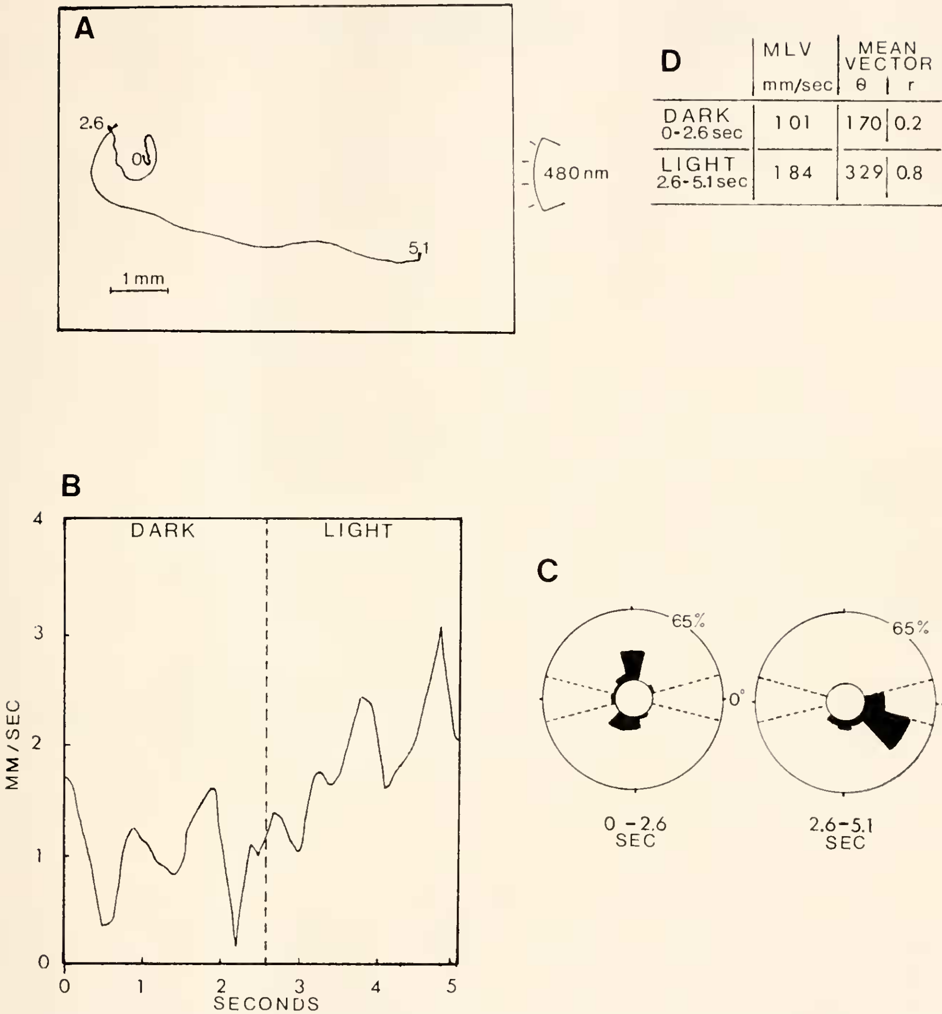

FIGURE 1. An example of computer analysis of a single *Balanus improvisus* nauplius. Digital processing of a video tape produces a computer tracking of the nauplius (A). A light stimulus (480 nm, 0.06 W/m^2) was applied from 2.6 to 5.1 sec. Linear velocity (B) and direction of travel (C) are calculated at 0.1-sec intervals (stimulus at 0°) and mean values for desired time intervals (D) determined.

Light-adapted stage II nauplii (15° C, 20‰) were exposed to 480 nm light at five intensities ranging from 27 to 0.0027 W/m^2. The initial MLV of nauplii exposed only to 830-nm darkfield illumination does not vary significantly ($P = 0.05$) between sample group (Table I). During light stimulation, the MLV for groups at the upper three light intensities increases significantly; recovery to MLV statistically equal to control levels does not occur within the three sec following stimulation. No significant change in MLV occurs at the lower two light intensities

(Table I). Plotting the running mean velocities of each sample group at 0.1-sec intervals shows the time sequence of naupliar photokinetic response (Fig. 2). The change in MLV is delayed in onset and return to normal, relative to light stimulus duration.

A change in MLV (orthophotokinesis) is observed only above 0.027 W/m². However a directional response to light (phototaxis) occurs at all intensities (Table I, Fig. 3). DOT distributions during the initial "dark" interval do not vary significantly ($P = 0.05$) from a theoretical uniform distribution. During the "light" interval, nauplii at all light intensities exhibit nonrandom distributions and a significant difference from initial distributions. Computer plotted histograms (Fig. 3) illustrate a directed response toward the light source.

The individual nauplii paths (*e.g.* Fig. 1A) and time analysis of MLV responses (Fig. 2) indicate that a delay of about 0.5 sec often occurs between stimulation and naupliar response. The DOT distribution for the entire 2.5-sec light stimulus interval clearly indicates a general positive phototactic movement (Fig. 4A). If the initial 0.5-sec "orientation period" at light stimulation is omitted from DOT determinations, a strong directed response becomes evident (Fig. 4B). For the following experiments, mean naupliar direction and MLV during the latter two sec of a 2.5-sec light stimulus are presented. Positive phototaxis is considered a mean direction of travel ± 15° of the light source; negative phototaxis is a DOT ± 15° in the opposite direction. Comparative results using a ± 45° "windows" in respective directions are also included.

To correlate with other laboratory studies (Lang, *et al.*, 1979; Lang, Miller, Lawrence, Marcy, and Clem, in progress), light intensity and spectral experiments were conducted at 20° C, using a tungsten light source and filters. Essentially the same positive phototaxis to 480-nm light was found using the new light source and at the higher temperature.

TABLE 1

Mean linear velocity and χ^2 directional response comparison of light-adapted stage II Balanus improvisus *nauplii (15° C 20‰) during initial "dark" interval (830-nm substage light) at 0–2.5 sec, 480-nm light stimulation at 2.5 to 5.0 sec, recovery "dark" interval at 5.0 to 8.0 sec. A null hypothesis that the distribution of direction of travel means* (60° intervals) *for each sample group of 30 to 38 nauplii is equal to a theoretical uniform distribution was tested using the χ^2 test. χ^2 values for $P \leq 0.05$ are indicated by an asterisk where P represents the probability of rejecting the null hypothesis when actually true.*

Intensity W/m² at 480 nm	Mean linear velocity mm/sec			Direction of travel Chi-square: experimental *vs.* uniform distribution		
	Initial "dark"	Light stimulation	Recovery "dark"	Initial "dark"	Light stimulation	Recovery "dark"
27	1.35 ± 0.35	2.13 ± 0.65	1.97 ± 0.63	7.14	33.67*	8.80
2.7	1.54 ± 0.55	2.29 ± 0.66	1.91 ± 0.61	4.43	64.14*	5.00
0.27	1.25 ± 0.39	1.82 ± 0.52	1.66 ± 0.55	7.86	29.81*	15.29*
0.027	1.46 ± 0.36	1.60 ± 0.45	1.55 ± 0.48	5.00	61.50*	8.74
0.027	1.46 ± 0.31	1.51 ± 0.38	1.50 ± 0.36	4.42	19.60*	8.47

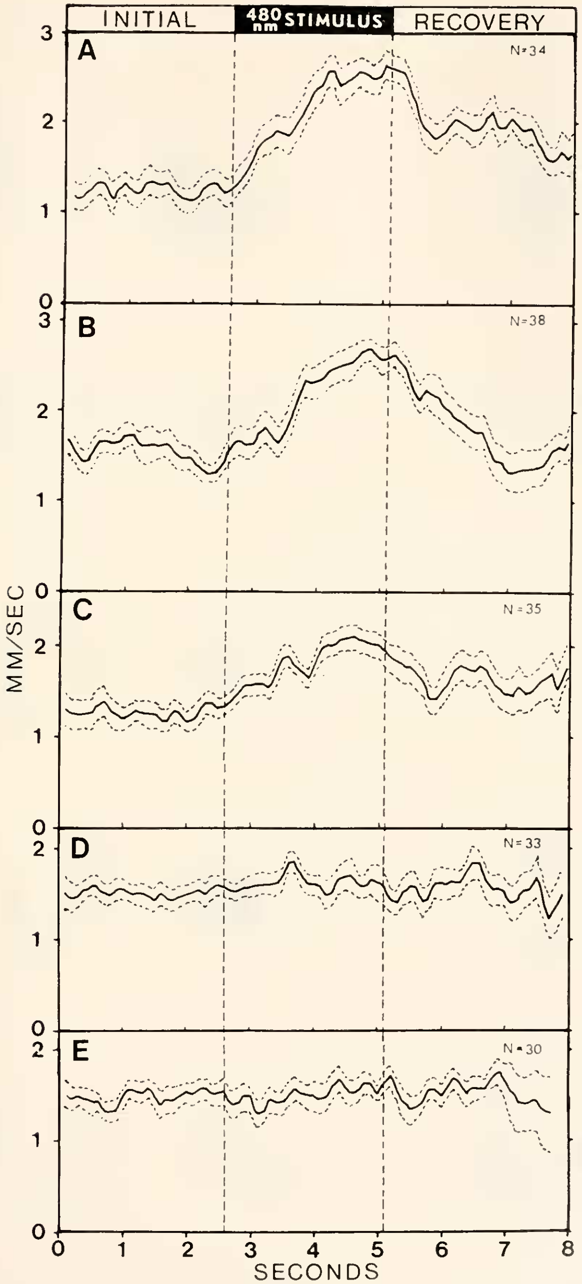

FIGURE 2. The running mean linear velocity ± variance of stage II *Balanus improvisus* nauplii in response to a 480-nm light stimulus applied at 2.6 to 5.1 sec: (A) 27 W/m^2; (B) 0.27 W/m^2; (C) 0.027 W/m^2; (D) 0.0027 W/m^2; (E) 0.00027 W/m^2. The number of naupliar paths analyzed (N) is indicated. Initial and recovery represent "dark" periods of 830-nm substage illumination only.

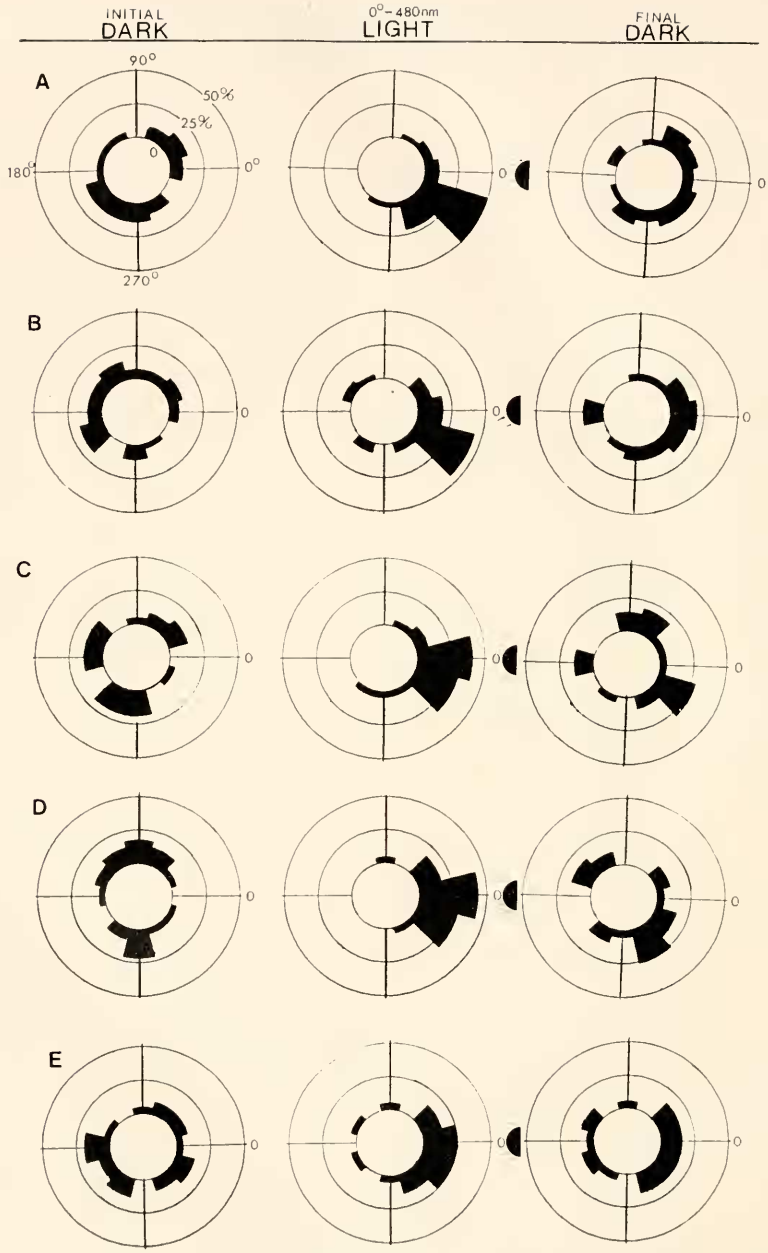

FIGURE 3. Distribution of mean direction of travel of stage II *Balanus improvisus* during initial 2.5-sec "dark" interval, 2.5 sec 480-nm light stimulus at 0°, and final 3.0-sec "dark" interval: (A) 27 W/m²; (B) 2.7 W/m²; (C) 0.27 W/m²; (D) 0.027 W/m²; (E) 0.0027 W/m². Sample numbers and "dark" condition are as in Figure 2. Significantly ($P \leq 0.05$) nonrandom distributions are indicated in Table I.

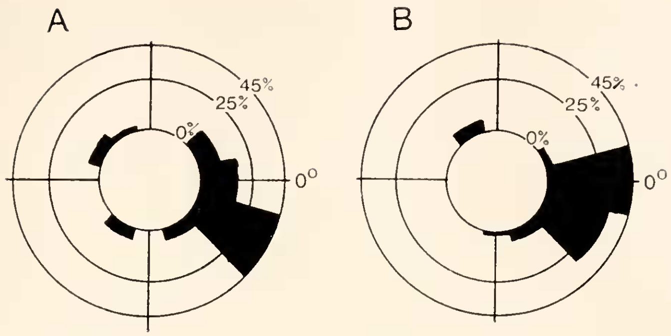

FIGURE 4. Distribution of mean direction of travel for 38 stage II *Balanus improvisus* exposed to a 480 nm, 2.7 W/m^2 light stimulus at 0° : (A) directional response during full 2.5-sec stimulus; (B) directional response during the latter 2.0 sec of the same stimulus.

Stage II response spectrum

Dark-adapted stage II nauplii (20° C, 15‰) were exposed to filtered light from 440 to 640 nm in 20-nm increments. Quantal intensity was balanced to approximately 0.07×10^{16} quanta m^2 sec at each wavelength (calculated values ranged

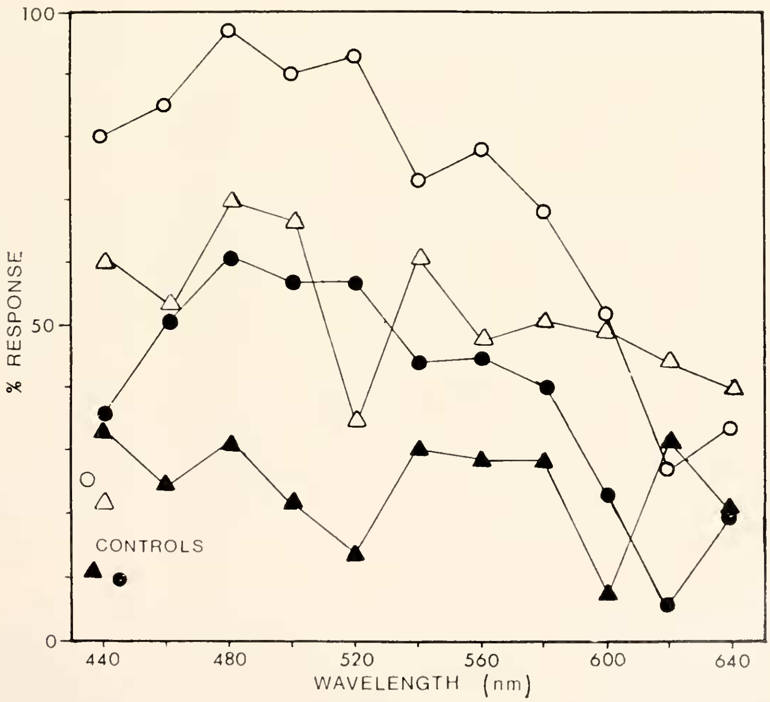

FIGURE 5. Response spectrum for positive phototaxis (% response). Open circle, stage II *Balanus improvisus*; response ± 45° of light source; closed circle; ± 15° of light source. Open triangle, stage VI *B. improvisus*; ±45° of light source; closed triangle, ±15° of light source. See Table II for sample numbers.

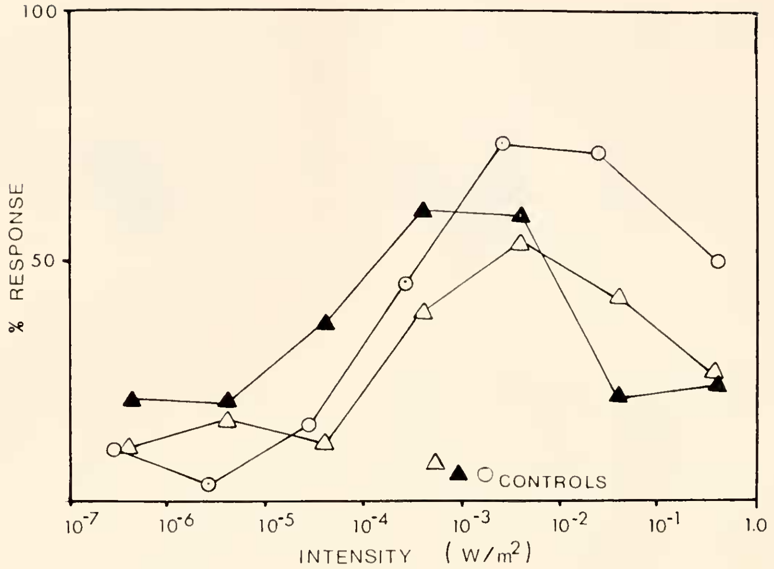

FIGURE 6. Intensity response for positive phototaxis of stage II *Balanus improvisus* at 480 nm. Closed triangle, dark-adapted at 20° C, 15‰ (N = 26–47); open triangle, light-adapted at 20° C, 15‰ (N = 53–60); open circle, light-adapted at 20° C, 30‰ (N = 22–29).

from $0.062–0.088 \times 10^{16}$). This quantal intensity was chosen because at 480-nm light it produced a clear positive phototactic response, but did not evoke a maximal response.

Analysis of DOT for nauplii indicates a broad response spectrum (Fig. 5). Increasing the ± 15° "window" for positive response to ± 45° produces a similar curve at response levels 30 to 40% higher. The peak percentage of positive phototaxis occurs at 480 nm (60% ± 15°; 97% ± 45°), however similar strong responses are evident at 500 to 520 nm. A significant negative response did not occur at any wavelength.

Although peak swimming speeds were found between 460 and 520 nm, distinct differences in MLV relative to wavelength are absent. One way analysis of variance followed by the Duncan test ($P = 0.05$) yields three broadly overlapping homogenous subsets, where the difference in the means of any two groups within a subset is not significant (Table II).

Intensity response

Light- and dark-adapted stage II nauplii (20° C, 15‰) were exposed to seven intensities of 480-nm light. In both groups, response to light was either positive or not evident; negative phototaxis did not occur significantly above random predictions (Fig. 6).

In close agreement with initial results, light-adapted nauplii exhibit a significant MLV increase at light intensities above 10^{-3} W/m^2 (Table III). Dark-adapted nauplii significantly increase MLV above 10^{-4} W/m^2 and, in contrast to

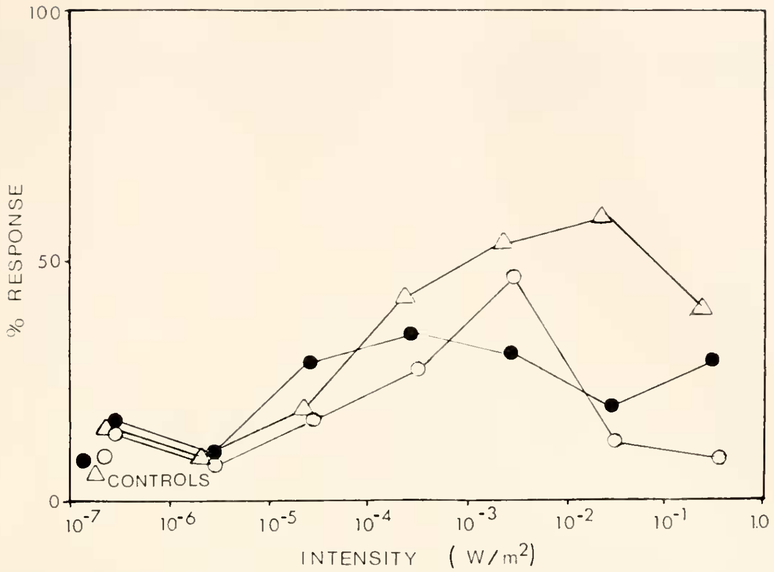

FIGURE 7. Intensity response for positive phototaxis of stage III and stage VI *Balanus improvisus* at 480 nm. Open triangle, light-adapted stage III at 20° C, 30‰ (N = 22–29); open circle, light-adapted stage VI at 20° C, 30‰ (N = 25–42); closed circle, dark-adapted stage VI at 20° C, 30‰ (N = 46–56).

light-adapted larvae, dark-adapted larvae show no increase in MLV at full intensity (Table III).

Directional response results further demonstrate the effects of dark adaptation. Light-adapted nauplii show a plateau of strong positive phototaxis from 10^{-3} to 10^{-1} W/m²; a similar pattern occurs from 10^{-4} to 10^{-2} W/m² for dark-adapted nauplii (Fig. 6).

A significant positive phototactic response is present at all intensities tested for dark-adapted larvae. For light-adapted larvae, no significant response occurs below 10^{-4} W/m². In respect to percent response and MLV, dark-adapted nauplii appear to have a somewhat "stronger" response to favorable light intensities relative to light-adapted nauplii (Fig. 6).

Some light-adapted stage II nauplii were also investigated at 30‰, 20° C, to permit comparison with later stage nauplii tested at higher salinity. A similar, but perhaps enhanced response, relative to results at 15‰ was observed (Fig. 6). The change in salinity does not alter the basic pattern of response in stage II nauplii.

Light response in later stage nauplii

Smaller numbers of stage III and stage VI nauplii were tested for response to light, in addition to stage II nauplii. The later stage *B. improvisus* larvae are likely to be carried into increased salinity water (Bousfield, 1955). Hence, these initial studies were conducted at 30‰.

Phototactic response of light-adapted stage III naupli (30‰, 20° C) is similar to stage II nauplii (Fig. 7). The control MLV of stage III nauplii tends

TABLE II

Mean linear velocities of stage II and stage IV Balanus improvisus *nauplii in response to 2.5-sec stimuli of light at different wavelengths. Homogeneous subsets as determined by the Duncan's multiple range test ($P = 0.05$) are indicated (see Nie,* et al., *1975).*

Wavelength $7.0–9.0 \times 10^{14}$ quanta/m²	Stage II			Stage VI		
	N	MLV ± s.d. mm/sec	subset	N	MLV ± s.d. mm/sec	subset
440	26	1.75 ± 0.66	BC	35	3.72 ± 1.35	A
460	46	1.97 ± 0.77	AB	33	3.21 ± 1.19	AB
480	40	1.85 ± 0.83	ABC	35	3.36 ± 1.38	AB
500	45	2.02 ± 0.99	AB	35	3.26 ± 1.49	AB
520	45	2.13 ± 0.79	A	40	2.94 ± 1.07	AB
540	40	1.92 ± 0.62	ABC	37	2.83 ± 1.51	B
560	34	1.69 ± 0.75	BC	34	2.80 ± 1.37	B
600	50	1.56 ± 0.60	C	36	3.05 ± 1.37	AB
620	34	1.81 ± 0.60	ABC	37	3.16 ± 1.18	AB
640	55	1.78 ± 0.66	BC	48	2.89 ± 1.18	B

to be greater than stage II (Table III). Stage III nauplii show no significant increase in MLV during any light stimulus tested (Table III) therefore the photokinetic response seen in stage II nauplii is absent.

Both the response spectrum and responses to different intensities were tested for stage VI nauplii. Photo-responsiveness of stage VI larvae is generally less than that observed for stage II and III larvae; individual variation in nauplii is noticeably increased.

The spectral response function of stage VI nauplii using a ± 15° "window" for positive photo-response yields a generally low response of about 25 to 35% at all wavelengths, except 520 and 600 nm (Fig. 5). Increasing the window to ± 45° produces a curve similar to stage II with peak response at 480 nm, however, responsiveness is 20% or more below stage II results (Fig. 5). Decreased responsiveness of stage VI nauplii at 520 nm and increased responsiveness above 600 nm relative to stage II larvae is evident (Fig. 5). Unlike stage II results, increasing the window to ± 45° changes the basic shape of the response curve for stage VI nauplii and is perhaps indicative of less precise orientation to the light stimulus during the 2.5-sec test interval.

The response of stage VI nauplii to different intensities of 480-nm light is most interesting in respect to dark adaptation. Light-adapted stage VI nauplii first show a significant phototactic response above 10^{-4} W/m² (Fig. 8), which is in agreement with stage II results (Fig. 7). However, unlike stage II nauplii, the positive phototaxis of stage VI nauplii rapidly diminishes above 10^{-2} W/m². Dark adaptation of stage VI nauplii, although enhancing phototaxis between 10^{-5} and 10^{-4} W/m² (Fig. 7), does not appreciably alter the threshold of response. Finally, in marked contrast to stage II (Fig. 7), dark adaptation does not decrease responsiveness of stage VI nauplii to higher light intensities (Fig. 7).

The MLV of stage VI nauplii did not vary significantly as a function of light intensity or wavelength (Table II, III). The MLV for all samples (3.05 mm/sec) was significantly above stage II and III results.

TABLE III

Mean linear velocities of Balanus improvisus *naupliar stages II, III, and VI in response to 2.5 sec 480-nm light stimuli of different intensities. Control represents swimming speeds with 830-nm sub-stage illumination. Homogeneous subsets as determined by the Duncan's multiple range test* ($P = 0.05$) *are indicated for each naupliar test group* (*see Nie,* et al., *1975*).

Intensity	Dark-adapted II			Light-adapted II		
	N	MLV ± s.d.	subset	N	MLV ± s.d.	subset
6.2×10^{-1}	32	1.63 0.84	BC	60	1.91 0.81	A
$\times 10^{-2}$	33	2.22 0.96	A	59	1.92 0.88	A
$\times 10^{-3}$	26	2.11 1.10	A	58	1.77 0.65	AB
$\times 10^{-4}$	37	1.94 0.73	AB	59	1.58 0.60	BC
$\times 10^{-5}$	36	1.55 0.588	C	60	1.44 0.51	C
$\times 10^{-6}$	47	1.54 0.51	C	54	1.63 0.66	BC
$\times 10^{-7}$	39	1.41 0.57	C	53	1.54 0.53	BC
Control	40	1.52 0.55	C	55	1.48 0.49	C

Intensity	Light-adapted III			Dark-adapted VI		
	N	MLV ± s.d.	subset	N	MLV ± s.d.	subset
4.5×10^{-1}	24	2.14 0.86	A	50	2.94 1.52	A
$\times 10^{-2}$	29	2.40 0.70	A	50	3.00 1.61	A
$\times 10^{-3}$	25	2.32 0.81	A	49	3.03 1.74	A
$\times 10^{-4}$	22	2.24 0.87	A	47	3.15 1.84	A
$\times 10^{-5}$	24	2.18 0.90	A	46	2.60 1.29	A
$\times 10^{-6}$	24	2.23 0.88	A	56	3.00 1.50	A
$\times 10^{-7}$	23	2.08 0.96	A	49	3.08 1.79	A
Control	23	2.20 0.73	A	50	2.98 1.25	A

DISCUSSION

The photophysiology of zooplankton has been recently reviewed by Forward (1976b). In general, zooplankton living in coastal and fresh water tend to have their primary spectral maximum in the 500- to 600-nm region, the wavelengths best transmitted in these waters. Visscher and Luce (1928) noted maximal response of *B. amphitrite* and "*B. improvisus*" cyprids at 530 to 545 nm (although reported as *B improvisus* cyprids, the time of collection and similarity in size to *B. amphitrite* cyprids suggest *Balanus eburneus* as the more probable species tested (McDougall, 1943; Lang, 1979)). In more precisely controlled experiments using stage I *E. modestus* and *S. balanoides* nauplii, Barnes and Klepal (1972) found maximal spectral sensitivity between 520 and 530 nm, with a marked shoulder of strong responses at 450 to 530 nm.

Stage II *B. improvisus* nauplii have a maximal spectral sensitivity at 480 to 520 nm, somewhat shorter wavelengths than expected considering their estuarine habitat and other previous findings for barnacle larvae. Strong photo response occurs within this spectral range with maximal positive phototaxis at 480 nm (Fig. 6) and maximal MLV response to light stimuli at 520 nm (Table II).

Light response studies of stage VI nauplii are hindered by high individual variability of responsiveness; some nauplii appear highly sensitive to light stimuli while others are unresponsive. Those stage VI nauplii which do respond show strong sensitivity to 440 to 500 nm, are curiously less sensitive to 520-nm light, and appear to be more sensitive to wavelengths at and above 540 nm relative to stage II (Fig. 6). Considerable structural and, presumably, physiological changes occur in stage VI nauplii in preparation for metamorphosis to cyprid (Walley, 1969). This includes development of compound eyes under the dorsal cephalic shield. Stage VI nauplii may have, depending on their age, only a single median naupliar eye, or two well pigmented compound eyes (Kaufmann, 1965; Lang, 1979). Since the larval samples tested in this study included all phases of stage VI development, variability of responsiveness is not unexpected. Increased sensitivity to higher wavelengths is perhaps related to development of compound eyes; studies on specific phases of stage VI development and, more importantly, the cyprid stage, are needed to verify this hypothesis.

Contrasting qualitative observation of photokinetic responses in barnacle larvae have been reported in the literature. Ewald (1912) noted a charatceristic sinking reaction in stage II specimens of *Balanus perforatus* following a sudden dark-to-light transition. Similarly Crisp and Ritz (1973) saw decreased activity in *S. balanoides* cyprids exposed to sudden light increase and conversely, increased activity following light intensity reduction. Essentially opposite reactions were observed in our laboratory for stage II *Balanus venustus, B. improvisus,* and *B. amphitrite;* MLV sharply increased following sudden exposure to bright white light and MLV sharply decreased following removal of the light stimulus (Lang, *et al.,* 1979). Photokinetic responses were found absent in stage II nauplii of *S. balanoides* and *E. modestus* (Crisp & Ritz, 1973) and in *Chthamalus fragilis* (Lang, *et al.,* 1979).

The present study shows that stage II nauplii of *B. improvisus* consistently increases MLV following exposure to a specific range of light intensities at 480 nm, the range being determined, in part, by the initial state of naupliar light adaptation. Dark-adapted nauplii show photokinetic response at reduced light intensities relative to light-adapted nauplii. The upper intensity, if any, which inhibits increased MLV in light-adapted nauplii was above the maximum tested (27 W/m^2). An increase in MLV appears to represent a second aspect in stage II light response. At detectable, but suboptimum intensities, stage II nauplii exhibit positive phototaxis but show no significant change in MLV. Only within a narrower range of light intensities inducing maximal response does MLV increase. A similar change in MLV does not occur in light-adapted stage III nauplii or light- and dark-adapted stage VI nauplii. Results indicate at least two possibilities; either stage II nauplii exhibit a "stronger" response to light incorporating both directional and kinetic reactions or, later stages normally swim at or near their potential MLV and are incapable of further MLV increase in response to light stimulii.

Earlier studies (Lang, *et al.,* 1979) showed that *B. improvisus* nauplii will briefly stop or reduce MLV when a strong white light source was removed. Removal of the 480-nm light stimulii in present studies did not induce this response. We assume this response occurs only with more intense and/or longer light stimulation.

The level of previous adaptation to light has been shown to significantly effect the photoresponse of zooplanktons (Forward, 1976b). Crisp and Ritz (1973) demonstrated relative reductions in barnacle naupliar light responsiveness following exposure to strong light. Continual exposure to light of sufficient intensity will induce a photonegative response in barnacle nauplii (Groom and Loeb, 1890).

A change in responsiveness to light following dark-adaptation is clearly seen here in *B. improvisus* stage II nauplii. The threshold for positive phototaxis in light-adapted nauplii is at least an order of magnitude greater relative to dark-adapted naupliar responses. Conversely, light-adapted phototaxis is significantly less suppressed at higher light intensities (Fig. 6). Positive phototaxis for at least 10% (above control) of dark-adapted stage II nauplii tested was seen at 6.2×10^{-7} W/m^2 at 480 nm. A consistent, strong response (*i.e.*, 30% or more of the test population) occurred above 10^{-5} W/m^2. These values bracket the same order of magnitude for light responsiveness reported by Barnes and Klepal (1972) and Crisp and Ritz (1973) for three barnacle species of naupliar stages I–II.

A similar clear shift in responsiveness of light- and dark-adapted stage VI nauplii was not seen. In particular, dark-adapted nauplii remained equally or more responsive to higher light intensities as compared to light-adapted nauplii (Fig. 7). Cyprid larvae of some barnacles are known to exhibit at least two fundamental light responses; orientation at fixation and shade-seeking during exploration (Crisp and Ritz, 1973). As with spectral results, we would eventually like to demonstrate whether idiosyncrasies in stage VI light behavior relate to light responses in *B. improvisus* cyprids.

The degree of light-adaptation, although it affects the intensity sensitivity of *B. improvisus* nauplii, does not alter the basic types of response. Under the stable and presumably favorable salinity-temperature conditions tested, the immediate reaction of *B. improvisus* to light stimulation is either positive phototaxis or no response. A characteristic "shadow response" with negative phototaxis seen in light-adapted brachyuran zoea (Forward, 1976a, 1977) tested under similar light stimuli is absent.

In his classic field study, Bousfield (1955) showed that stage II *B. improvisus* nauplii maintained an average water column position near the surface, while later naupliar stages were found at progressively lower average depths. The strong positive photo response of stage II nauplii and marked decline of photo responsiveness in stage VI nauplii seen here correlates well with these field observations. However, a discussion of ecological implications of larval photobehavior is best deferred until further studies are complete.

This study most importantly demonstrates a new research technique. Video-computer systems decidedly enhance the ability to convert visual (video) records into quantified data. With this ability comes a new potential to initiate studies on locomotory behavior involving various parameters and to measure responses with a resolution previously not readily obtainable.

Additional software development for analysis of phototactic responses was provided by Dr. Robert Wilson. Technical assistance for Bugwatcher operations was provided by Dr. John Greaves. Martha Marcy and Sally Lawrence contributed significant assistance in larval maintenance and data analyses.

Summary

A video-computer behavioral analysis system—the "Bugwatcher"—was found to be capable of rapidly and accurately analyzing the phototactic movements of stage II, III, and VI nauplii of the barnacle *Balanus improvisus*. Under the test conditions these larvae only display a positive phototactic response; a negative response was not observed. The response spectrum of dark-adapted stage II shows a plateau of strongest positive phototaxis between 480 and 520 nm with about 60% of test larvae swimming $\pm 15°$ toward the light stimulus. In contrast, stage VI nauplii are generally less phototactic. The response spectrum changes to have a depression at 520 nm and enhanced responsiveness to longer wavelengths of 540 to 580 nm.

Responsiveness to different light intensities changed upon light adaptation. Upon stimulation with 480 nm lights, dark-adapted stage II nauplii show a significant positive phototaxis at 6.2×10^{-7} W/m². Peak response occurs between 10^{-4} and 10^{-2} W/m². A signficant increase in mean linear velocity (MLV) accompanies the maximal phototactic response. In contrast, upon light adaptation, stage II nauplii show a significant positive phototaxis only to intensities above 10^{-4} W/m², 480 nm. Peak response occurs between 10^{-3} and 10^{-1} W/m². A significant increase in MLV occurs from about 10^{-3} to at least 27 W/m². Light-adapted stage III nauplii show intensity sensitivity similar to stage II nauplii; however, no increase in MLV occurs during light stimulation. Stage VI nauplii show a reduced percent phototactic response at all intensities as compared to stage II–III. Light- or dark-adaptation does not result in clear shifts in intensity sensitivity as evident in stage II responses. Differences in stage VI naupliar light responses may be related to impending metamorphisis to cyprid. A study of cyprid light response is needed to clarify this.

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